

## The thiol-disulfide balance in smokers and non-smokers

Thiol/disulfide balance in smokers

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### Abstract

**Aim:** The impairment of thiol/disulfide balance plays a critical role in the development and/or progression of many diseases. This study aims to investigate the effects of smoking on dynamic thiol-disulfide balance as a marker of oxidative stress in healthy individuals. **Material and Method:** The study included 30 smoking and 30 non-smoking healthy individuals. The age, gender, body mass index (BMI) and smoking habits of all of the participants were recorded, and thiol-disulfide balance tests, including total thiol (TT), native thiol (NT) and disulfide (DS), were determined using a novel colorimetric method. **Results:** The TT and NT levels of the smokers were significantly lower than those of the non-smokers, whereas there were no significant differences in the DS levels and DS/NT, DS/TT and NT/TT ratios of the two groups. In the smoking group, BMI was negatively correlated with NT and TT levels and NT/TT ratio and positively with DS/NT and DS/TT ratios. Age was negatively correlated with NT and TT levels in the smokers. **Discussion:** Our findings demonstrated that NT and TT levels were reduced in smokers. We believe that supplementation of thiol-containing compounds may diminish the harmful effects of smoking by preventing or alleviating smoking-induced oxidative stress.

### Keywords

Smoking; Oxidative stress; Thiol-Disulfide Balance

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## Introduction

Tobacco smoke contains high levels of free radicals and other oxidants, in addition to toxins and carcinogens [1], and is an important risk factor in the development of pathological conditions such as chronic obstructive pulmonary disease [2], lung cancer [3] and coronary heart disease [4]. Smoking increases oxidative stress both through the increased production of free radicals and the weakening of the antioxidant defense systems [5,6] and so leads to oxidative damage to such biological structures as nucleic acids, lipids and proteins [7].

Thiols are organic molecules containing a sulfhydryl group (–SH) that play crucial roles in the defense mechanism against oxidative stress [8]. Under conditions of oxidative stress, thiol groups can easily be oxidized and form reversible disulfide (DS) bonds, and these formed DS bonds can later be reduced back to thiol groups [9]. This oxidation-reduction cycle between the thiol and DS groups ensures the maintenance of a dynamic thiol-disulfide balance, which is essential for several physiological processes, including, but not limited to, cell signaling, xenobiotics detoxification and antioxidant defense [10,11].

Recent studies have demonstrated that changes in the thiol-disulfide balance are associated with the etiology and/or progression of various diseases [12–15]. This study aims to investigate the effects of smoking on dynamic thiol-disulfide balance in healthy individuals.

## Material and Method

This study included 30 (8 female/22 male) smoking and 30 (9 female/21 male) non-smoking healthy individuals with body mass indexes (BMI) of 20–30 kg/m<sup>2</sup>, all of which were personnel of the Harran University Medical Faculty. The age, gender, BMI and smoking habits (including duration of smoking and number of cigarettes smoked per day) were recorded for all participants. Individuals with a systemic disease, acute/chronic infections, and those using alcohol or receiving antioxidant and vitamin supplement therapies, were excluded from the study. The study was approved by the Harran University Ethics Board Research Commission.

## Specimen collection and analysis

Fasting blood samples were collected from all participants into tubes containing ethylenediaminetetraacetic acid for the measurement of thiol/disulfide balance tests.

The collected blood samples were immediately centrifuged at 3000 rpm for 10 minutes, and the plasma was separated and kept at -80°C until the time of analysis. The collected samples were stored under similar conditions, and following similar processes.

Plasma total thiol (TT) and native thiol (NT) concentrations were determined by the new method by Erel and Neselioglu [16]. According to this method, reducible DS bonds are first reduced to functional thiol groups. In the second stage, the sodium borohydride that was used as a reducing agent was removed with formaldehyde, after which, 5,5'-Dithiobis (2-nitrobenzoic acid) was used to detect all thiol groups (reduced thiol and native thiol). Dynamic DS concentrations were determined using the (TT-NT)/2 formula, and the (DS x100)/NT, the (DS x100)/TT, and (NTx100)/TT ratios were calculated. Albumin levels were deter-

mined using the Bromocresol green method in a Cobas c501 analyzer (Roche Diagnostics, Germany).

## Statistical Analysis

The statistical analysis was carried out using the SPSS 21 (IBM Corp, Armonk, NY) program and the level of significance was accepted as  $p < 0.05$ . A Shapiro–Wilk test was used to identify the level of normality in the distribution of data. Continuous variables were compared between the two groups using an independent sample t-test or a Mann-Whitney U-test, and the results were presented as the mean  $\pm$  sd or median (min-max). A Chi-Square test was used to compare categorical variables, and the results were expressed as numbers and percentages. The relationship between the thiol-disulfide balance tests and other variables (including age, BMI and pack-years of cigarettes smoked) were analyzed with Spearman and Pearson correlation tests.

## Results

The demographic characteristics of the smokers and non-smokers are summarized in Table 1. There was no statistical difference between the two groups in terms of age, gender distribution or BMI. TT and NT levels were significantly lower in smokers than in non-smokers ( $p = 0.002$  for both). The DS levels, and DS/NT, DS/TT and NT/TT ratios were similar between the two groups ( $p > 0.05$  for all) (Table 2).

In the smoking group, BMI negatively correlated with NT ( $r = -0.416$ ,  $p = 0.022$ ), TT ( $r = -0.380$ ,  $p = 0.038$ ) levels and NT/TT ratios ( $r = -0.446$ ,  $p = 0.014$ ), and positively correlated with DS/NT ( $r = 0.442$ ,  $p = 0.014$ ) and DS/TT ( $r = 0.446$ ,  $p = 0.014$ ) ratios. Also, age was negatively correlated with NT ( $r = -0.468$ ,  $p = 0.009$ ) and TT ( $r = -0.491$ ,  $p = 0.006$ ) levels in the smokers. However, there was no correlation between thiol-disulfide balance tests and the number of pack-years of cigarettes smoked ( $p > 0.05$ ).

## Discussion

Tobacco smoke, which contains high levels of free radicals and other oxidants, increases oxidative stress by causing an oxidant/antioxidant imbalance [17]. Oxidative stress induced

Table 1. Characteristics of the study population.

	Smoker (n=30)	Non-smoker (n=30)	p Value
Age (years)	29 (18–40)	25 (18–45)	0.366
Gender (male), n (%)	22 (73.3)	19 (63.3)	0.405
Body mass index (kg/m <sup>2</sup> )	24.6 $\pm$ 3.2	23.5 $\pm$ 2.5	0.139
Cigarette pack-years	10.4 $\pm$ 8.1	-	-

Table 2. Plasma levels of thiol/disulfide balance tests of the study population.

	Smoker (n=30)	Non-smoker (n=30)	p Value
Albumin, g/dL	4.49 $\pm$ 0.26	4.59 $\pm$ 0.16	0.081
Native thiol (NT), $\mu$ mol/L	357.3 $\pm$ 61.9	409.2 $\pm$ 64.4	0.002*
Total thiol (TT), $\mu$ mol/L	403.9 $\pm$ 65.8	458.6 $\pm$ 69.1	0.002*
Disulfide (DS), $\mu$ mol/L	23.3 $\pm$ 4.5	24.3 $\pm$ 5.9	0.361
Disulfide/native thiol (DS/NT), %	6.6 $\pm$ 1.4	6.1 $\pm$ 1.6	0.215
Disulfide/total thiol (DS/TT), %	5.8 $\pm$ 1.1	5.4 $\pm$ 1.3	0.203
Native thiol/total thiol (NT/TT), %	88.3 $\pm$ 2.2	89.1 $\pm$ 2.6	0.204

\* $p < 0.05$ .

by smoking has been shown to be associated with various diseases, including respiratory disorders, cardiovascular diseases and cancer [18]. Thiols are important antioxidants that protect cells and tissue from oxidative damage. Low molecular weight thiols (i.e., glutathione cysteine, cysteinylglycine), albumin and other protein thiols form the thiol pool of the body [19]. Moriarty et al. [20] measured the levels of glutathione (GSH) and its oxidized DS form (GSSG), as well as cysteine (Cys) and its oxidized DS form cystine (CySS), as markers of oxidative stress in smokers. They observed that the GSH/GSSG and Cys/CySS redox balances shift towards the DS form. Similar results were demonstrated by Diken et al. [21] in long-term smokers. In these studies, the thiol and disulfide levels of the low molecular weight thiols which account for a small part of the thiol pool were measured. Therefore, these studies may not reflect the exact thiol-disulfide status in plasma.

In 2014, thiol-disulfide balance can be assessed cumulatively by the novel method developed by Erel et al. which provides low-cost, practical, simple and reliable measurements [16]. There is only one study in the literature investigating the effect of smoking on dynamic thiol-disulfide balance in healthy individuals using this new method [22]. Solak et al. [22] showed recently that TT and NT levels were significantly lower and DS level, DS/TT and DS/NT ratios were significantly higher in smokers when compared to non-smokers. Similarly, the TT and NT levels of smokers in this study were significantly lower than in the controls. As smoking is closely associated with oxidative stress [5,6] and inflammation [23,24] smokers can be expected to have decreased levels of thiol. The thiols enter into reactions to neutralize smoking-mediated free radicals and ROS, which may reduce thiol levels.

Some researchers reported that N-acetyl cysteine, a thiol donor, reduces smoking-induced oxidative stress due to its antioxidant and anti-inflammatory effects [25,26]. Accordingly, in order to maintain a thiol/disulfide balance, using supplements containing N-acetylcysteine or other thiol donors may be considered a favorable intervention strategy against the harmful effects of smoking.

Contrary to the findings of Solak et al. [22], we did not find a significant difference between smokers and non-smokers in terms of DS level, DS/TT and DS/NT ratios. This can be explained with the inadequate number of participants in our study. This conflict in results may be also due to the differences in the duration and intensity of cigarette smoked among participants. In the literature, it has been documented that GSH and TT, and NT levels decreased with age [22,27]. Jones et al. [28] demonstrated that GSH/GSSG and Cys/CySS redox states were oxidized in association with increased age. In line with the findings of Babaoglu et al. [29], we observed a negative correlation between age and TT and NT levels in the present study. We also demonstrated that BMI in smokers correlated positively with DS/NT and DS/TT ratios, and inversely with NT, TT levels and NT/TT ratios, and similar results were reported by Elmas et al. [30]. On the other hand, mean age and BMI were similar between the smokers and non-smokers in our study, and so the potential impact of age and BMI on thiol-disulfide balance should be investigated in future studies.

There are some limitations in this study such as an insufficient

number of participants, its cross-sectional design, and the absence of elderly smokers. In this study, the TT and NT levels in smokers were found to be significantly lower than in non-smokers, and this suggests that thiols are important antioxidants that protect the organism from smoking-mediated free radicals and other oxidants. Accordingly, supplementation of thiol-containing molecules such as N-acetyl cysteine or alpha-lipoic acid may reduce the harmful effects of smoking by preventing or alleviating smoking-induced oxidative stress.

#### Scientific Responsibility Statement

*The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.*

#### Animal and human rights statement

*All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.*

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#### Conflict of interest

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